

Claims

1. Protein L having the ability to bind to the light chains of immunoglobulins, characterized in that the protein L has the following amino acid sequence:

— B1

Ala	Val	Glu	Asn	Lys	Glu	Glu	Thr	Pro	Glu	Thr	Pro	Glu	Thr	Asp	Ser
1				5					10					15	
Glu	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Phe	Ala	Asn	Gly	Ser
			20					25					30		
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Lys	Ala	Thr	Ser	Glu
		35					40					45			
Ala	Tyr	Ala	Tyr	Ala	Asp	Thr	Leu	Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr
	50					55					60				
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly
65	B2				70				75					80	
Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala
			85					90						95	
Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly
		100					105					110			
Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	Ala	Tyr	Arg	Tyr	Ala	Asp	Ala	Leu
		115					120					125			
Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr
	130					135					140				
Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro
145				150				155						160	
Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys
			165					170					175		
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu
		180					185						190		
Ala	Tyr	Arg	Tyr	Ala	Asp	Leu	Leu	Ala	Lys	Glu	Asn	Gly	Lys	Tyr	Thr
	195					200						205			
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly
210						215					220				

— B3

— B4  
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
 225 230 235 240  
 5 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
 245 250 255  
 Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu  
 260 265 270  
 10 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr  
 275 280 — B5 285  
 Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu  
 290 295 300  
 Glu

15

and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

2. DNA-sequence, characterized in that  
 20 it codes for the protein according to Claim 1 and has  
 the following nucleotide sequence:

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 28  
 25 GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC 96  
 ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 144  
 GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 192  
 30 GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240  
 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288  
 AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336  
 ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384  
 35 AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 432  
 ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480  
 AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576  
 GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624  
 5 GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672  
 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720  
 AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768  
 10 ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816  
 GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864  
 ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912  
 15 GAA TAATAA 921

3. A hybrid protein, characterized in  
 that it includes one or more of the B1-B5-domains ac-  
 20 cording to Claim 1 which bind to the light chains in  
 immunoglobulins of all classes, and domains which bind  
 to heavy chains in immunoglobulin G.

4. A hybrid protein according to Claim 3, characterized  
 25 in that the domains which bind to  
 heavy chains in immunoglobulin G are chosen from among  
 the C1- and C2-domains in protein G or from among any  
 other functionally similar proteins which bind to heavy  
 chains in immunoglobulin G, and variants, subfragments,  
 30 multiples or mixtures thereof having the same binding  
 properties.

5. A hybrid protein according to Claim 4, characterized  
 35 in that the hybrid protein has the  
 following amino acid sequence:

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser  
 5  
 Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser  
 20 25 30  
 Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu  
 35 40 45  
 10 Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr  
 50 55 60  
 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
 65 70 75 80  
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
 15 85 90 95  
 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
 100 105 110  
 Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu  
 115 120 125  
 20 Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr  
 130 135 140  
 Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro  
 145 150 155 160  
 25 Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys  
 165 170 175  
 Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu  
 180 185 190  
 Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr  
 195 200 205  
 30 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
 210 215 220  
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
 225 230 235 240  
 35 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
 245 250 255

[illegible]

30 and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

6. DNA-sequence, characterized in that  
it codes for a protein according to Claim 5 and has the  
35 following nucleotide sequence:

	GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA	48
	GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC	96
	ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA	144
	GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT	192
5	GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA	240
	AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA	288
	AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA	336
10	ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA	384
	AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT	432
	ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA	480
	AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA	528
15	ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA	576
	GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA	624
	GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA	672
20	AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA	720
	AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA	768
	ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA	816
	GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC	864
25	ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA	912
	GAA CCC ATG GAC ACT TAC AAA TTA ATC CTT AAT GGT AAA ACA TTG AAA	960
	GGC GAA ACA ACT ACT GAA GCT GTT GAT GCT GCT ACT GCA GAA AAA GTC	1008
30	TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT GAC GGT GAA TGG ACT TAC	1056
	GAC GAT GCG ACT AAG ACC TTT ACA GTT ACT GAA AAA CCA GAA GTG ATC	1104
	GAT GCG TCT GAA TTA ACA CCA GCC GTG ACA ACT TAC AAA CTT GTT ATT	1152
	AAT GGT AAA ACA TTG AAA GGC GAA ACA ACT ACT AAA GCA GTA GAC GCA	1200
35	GAA ACT GCA GAA AAA GCC TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT	1248
	GAT GGT GTT TGG ACT TAT GAT GAT GCG ACT AAG ACC TTT ACG GTA ACT	1296
	GAA ATG TAATAA	1308

7. DNA-sequence, characterized in that it codes for a protein according to Claim 3 and 4.

5 8. DNA-sequence, characterized in that it hybridizes to the DNA-sequence of Claim 2, 6 or 7 under conventional conditions and codes for a protein which has the same binding properties as the protein according to any one of Claims 1 and 3-5.

10 9. A plasmid vector, characterized in that it includes a DNA-sequence according to any one of Claims 2 and 6-8, preferably the vector pHDLG or pHDL.

15 10. A host cell, characterized in that it is transformed with the hybrid plasmid according to Claim 9, in particular a host which belongs to the species E. coli, particularly E. coli LE392, or Bacillus subtilis, Saccharomyces cerevisiae, preferably Id. Ref. DSSM E. coli LE392 pHDL and E. coli LE392/pHDLG respectively.  
20

11. A method for producing a protein according to Claims 1 and 3-5, characterized by cultivating a host cell according to Claim 10 under suitable  
25 conditions; accumulating the protein in the culture or lysing the cells and extracting the protein therefrom.

12. A reagent apparatus for binding, separating and identifying immunoglobulins, characterized  
30 in that it includes a protein according to any one of Claims 1 and 3-5.

13. A composition, characterized in that it includes a protein according to any one of Claims 1  
35 and 3-5, and optionally additives or carriers.

14. A pharmaceutical composition, c h a r a c -  
t e r i z e d in that it includes a protein according  
to any one of Claims 1 and 3-5, and optionally a pharma-  
ceutically acceptable carrier or extender.